What is claimed is:

1. A method of preparing a pro-urokinase ("pro-UK") mutant polypeptide, the method comprising

(a) obtaining a nucleic acid molecule that encodes a pro-UK mutant polypeptide;

(b) inserting the nucleic acid molecule into a pET29a expression plasmid comprising a phage T7 promoter and Shine-Dalgarno sequence;

(c) transforming *E. coli* type B strain bacteria BL21/DE3 RIL with the expression plasmid;

(d) culturing the transformed bacteria for a time and under conditions sufficient to enable the bacteria to express pro-UK mutant polypeptide; and

(e) isolating the pro-UK mutant polypeptide from the transformed bacteria.

2. The method of claim 1, wherein the pro-UK mutant is a pro-UK flexible loop mutant.

3. The method of claim 2, wherein the pro-UK flexible loop mutant comprises the mutation $Lys^{300} \rightarrow$ His.

4. The method of claim 1, wherein the pro-UK mutant is non-glycosylated and has a molecular weight of about 45,000 daltons.

5. The method of claim 1, wherein culturing comprises a two-stage fermentation.

6. The method of claim 5, wherein the first stage of fermentation comprises adding to a flask a cell culture diluted in sterile EC1 medium and growing the culture at about 34 to 37°C for at least about 10 hours with agitation to form a seed culture, wherein the cell culture comprises a glycerol suspension of an LB culture of the transformed bacteria and containing a sufficient amount of kanamycin.

7. The method of claim 5, wherein the second stage of fermentation comprises

a) adding the seed culture to a fermentor;

b) maintaining the pH in the fermentor at about 6.8 to 7.2;

c) maintaining the dissolved oxygen concentration in the culture medium at about 35 to 45% of air saturation;

d) maintaining the temperature of fermentation at about 34 to 37°C; and

e) adding to the fermentor a nutrient feeding solution comprising one or more sugars when all glucose initially present in the fermentor at step a) is consumed, following the equation $V = Vo\ e^{0.18t}$, where V = volume of feeding solution added (ml/h), Vo = 1/100 of the starting fermentation medium (ml), and t = time of fermentation after the start of the feeding phase (hours).

8. The method of claim 1, wherein the expression plasmid containing the nucleic acid molecule is pET29aUKM5.

9. The method of claim 1, further comprising preparing two-chain pro-UK mutant by passing the pro-UK mutant over plasmin bound to a substrate.

10. The method of claim 9, wherein the substrate is an agarose-based gel filtration medium.

11. The method of claim 1, further comprising combining the isolated pro-UK mutant polypeptide with an acidic excipient.

12. A composition comprising an isolated, single-chain pro-urokinase ("pro-UK") mutant polypeptide produced according to the method of claim 1, wherein at least 96% of the protein in the composition is the single-chain pro-UK mutant polypeptide.

13. A composition of claim 12, wherein at least 98% of the protein in the composition is the pro-UK mutant polypeptide.

14. The composition of claim 12, wherein the pro-UK mutant polypeptide is a pro-UK flexible loop mutant.

15. The polypeptide of claim 12, wherein the pro-UK mutant is M5.

16. The composition of claim 12, further comprising a pharmaceutically acceptable excipient.

17. A composition of claim 16, wherein the pharmaceutically acceptable excipient is an acidic excipient.

18. A composition comprising an aliquot of 20 to 40 mg of a pro-UK mutant made by the method of claim 1, packaged with directions for use in administering as a bolus to a patient exhibiting symptoms of a stroke or a heart attack.

19. A purified culture of *E. coli* type B strain bacteria BL21/DE3 RIL, wherein bacteria in the culture comprise an expression plasmid encoding a pro-urokinase flexible loop mutant polypeptide.

20. The culture of claim 19, wherein the expression plasmid is pET29aUKM5.

21. A method of preparing a pro-urokinase ("pro-UK") mutant polypeptide, the method comprising

(a) obtaining a transformed bacteria, wherein the bacteria is an E. coli type B strain bacteria BL21/DE3 RIL transformed with a pET29a expression plasmid comprising a phage T7 promoter, a Shine-Dalgarno sequence, and a nucleic acid molecule that encodes a pro-UK mutant polypeptide;

(b) culturing the transformed bacteria for a time and under conditions sufficient to enable the bacteria to express pro-UK mutant polypeptide; and

(c) isolating the pro-UK mutant polypeptide from the transformed bacteria.

22. The method of claim 21, wherein the pro-UK mutant is a pro-UK flexible loop mutant.

23. The method of claim 22, wherein the pro-UK flexible loop mutant comprises the mutation $Lys^{300} \rightarrow$ His.

24. The method of claim 21, wherein the expression plasmid containing the nucleic acid molecule is pET29aUKM5.